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The Effect of Controlling Temperature and Relative Humidity on Tyrophagus Putrescentiae (Schrank) (Sarcoptiformes: Acaridae) Infestations on Dry Cured Hams Treated in Food Grade Ingredient Infused Nets

Jasmine Deneen Hendrix

The effect of controlling temperature and relative humidity on *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae) infestations on dry cured hams treated in food grade ingredient infused nets

By

Jasmine Deneen Hendrix

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By

Jasmine Deneen Hendrix

Approved:

_____

M. Wes Schilling
(Major Professor)

_____

Taejo Kim
(Committee Member)

_____

Christine L. Cord
(Committee Member)

_____

Jerome Goddard
(Committee Member)

_____

Juan L. Silva
(Committee Member)

_____

Marion W. Evans, Jr.
(Graduate Coordinator)

_____

George M. Hopper
Dean
College of Agriculture and Life Sciences

Name: Jasmine Deneen Hendrix

Date of Degree: December 8, 2017

Institution: Mississippi State University

Major Field: Food Science

Major Professor: Dr. M. W. Schilling

Title of Study: The effect of controlling temperature and relative humidity on Tyrophagus putrescentiae (Schrank) (Sarcoptiformes: Acaridae) infestations on dry cured hams treated in food grade ingredient infused nets

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Candidate for Degree of Master of Science

Since methyl bromide is an ozone depleting substance, there is a significant need to find effective alternative compounds to control mite infestations on dry cured hams. Therefore, the objective of this study was to determine the most effective relative humidity and/or temperature to minimize mite reproduction and mold growth on dry cured hams in untreated and food grade ingredient infused nets. Mites on ham slices in untreated nets were reduced from the initial inoculum level of 50 mites per ham slice when exposed to 85% RH at 24, 28, and 32°C. Results indicated that hams should be stored at 85% RH or greater to minimize mite reproduction when xanthan gum and propylene glycol infused nets are used. Nets infused with carrageenan, propylene glycol alginate, and propylene glycol, completely inhibited mite reproduction at 85% RH and were effective at controlling mold growth.

DEDICATION

This thesis is dedicated back to my Lord and Savior, Jesus Christ. Thank you for using me as *Your* vessel to do *Your* good works. May *You* be glorified in all that I do.

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CHAPTER I

INTRODUCTION

Dry cured ham is produced from the hind leg of a pork carcass and is typically cured with a mixture of salt, nitrate, nitrite, and/or desired seasonings (Toldrá and Aristoy, 2010). The curing mixture promotes preservation and the development of the unique flavor and aroma of dry-cured ham (Zhao et al., 2016a; USDA FSIS 9 CFR 319.106). The ham may undergo additional flavoring through a smoking process and is aged until the ham loses at least 18% of its original weight and/or the desired product quality is obtained (USDA FSIS 9 CFR 319.106). The aging conditions of dry cured ham vary with the country of origin, length of processing, and desired flavor intensity.

Variance in aging conditions generally occur based on individual producer's preference and/or the country of origin in which the hams are cured (Andres et al., 2004). For example, dry cured hams that are produced in Europe are commonly aged at temperatures between 16 and 25°C and a relative humidity (RH) ranging between 65% and 85% (Toldrá and Aristoy, 2010). Comparatively, hams in the United States are commonly aged at higher temperatures, usually greater than 28°C (Rentfrow et al., 2012) and at similar RHs, producing hams with a firm texture and an intense salty flavor. However, these conditions also facilitate mold growth and are optimal conditions for pest development and reproduction on dry cured hams (Sanchez-Molinero et al., 2010).

Tyrophagus putrescentiae (Schrank) (Sarcoptiformes: Acaridae), also known as the ham or cheese mite, commonly infests stored food products such as dry cured hams. *T. putrescentiae* are commonly found in the environment, and reproduce readily at the environmental conditions which dry cured hams are aged. The high fat and protein content, intense flavor, moldy surfaces, and environmental temperature and humidity (20-30°C, 60-80% RH) during the aging process are factors that attract ham mites to dry cured ham. *T. putrescentiae* feed directly on the surface of the dry cured ham and the mold that grows on the ham's surface at these conditions, including *Aspergillus*, *Cladosporium*, and *Penicillium*. Mites may also penetrate the dry cured ham's surface, thus resulting in economic losses for dry cured ham producers.

Methyl bromide fumigation is the only known method that is used to effectively control ham mite infestations within the dry cured ham industry (Rentfrow et al., 2012). However, in 1992, methyl bromide was listed as an ozone depleting substance in the Montreal Protocol (UNEP, 1992), and is being phased out of industry use on an international scale. Hence, there is a significant need to find safe, economical, and effective alternative compounds and methods to replace the use of methyl bromide (Fields and White, 2002). Several researchers from universities and companies are pursuing alternative means of fumigation to control mite infestations, and have developed effective alternatives under laboratory conditions including phosphine fumigations, food grade ingredient coatings, and food grade ingredient infused nets (Sekhon et al., 2010a; Zhao et al., 2014; Abbar et al., 2016a; Campbell et al., 2016a; Zhang et al., 2017).

Phosphine has been tested as a potential alternative fumigant that was effective at controlling all mite development stages under laboratory conditions. However, it was ineffective in commercial settings due to the corrosion of copper wiring in the processing facilities (Zhao, Abbar, Phillips & Schilling, 2015). Propylene glycol (PG) has been incorporated into food grade coatings that are formulated in combination with xanthan gum, carrageenan, and propylene glycol alginate to control mite infestations on dry cured ham in benchtop experiments. Coatings with 50 % PG that were applied to ham cubes at 24°C and 50% RH were effective at controlling mite reproduction on dry cured ham under laboratory conditions (Zhao, Abbar, Phillips & Schilling, 2015).

Since coating whole hams generates additional processing steps, polyester and/or cotton blended nets, that are commonly used to hang dry-cured hams during the aging process, were infused with the coating mixture developed by Zhao et al. (2015) and evaluated for their efficacy at controlling mite infestations. The treated nets that contained at least 40% propylene glycol were effective at controlling mite reproduction on dry cured ham in benchtop studies (Abbar et al., 2016a; Campbell et al., 2016a). In addition, methods involving the manipulation of temperature and relative humidity have been investigated and have been observed for their effects on mite reproduction and development (Sánchez-Ramos et al., 2007). However, there is no research on the effect of temperature and relative humidity on *T. putrescentiae* infestations on dry cured hams that are placed in food-grade ingredient nets.

In the current study, temperature and relative humidity were evaluated as a physical means to control *T. putrescentiae* infestations on dry cured hams in food-grade

ingredient nets. *T. putrescentiae* mites are able to endure low and high temperatures ranging between 7-10°C and 35-38°C, respectively, but the ideal temperature range for maximum growth and development of *T. putrescentiae* mites is 25 to 33°C (Fields, 1992; Hubert et al., 2010; Collins, 2012; Zhao et al., 2016a). Research has indicated that 90% of all developmental stages of ham mites could be controlled via freezing, but freezing adversely affects ham quality (Rentfrow et al., 2006; Eaton and Kells, 2011).

Relative humidity is a critical constraint for aging dry cured hams. Dry cured hams are typically aged at RHs of 65 to 75% for 3 to 24 months in the United States. This provides optimal conditions for the growth and development of *T. putrescentiae* mites (Marriott et al., 1992). However, it was reported that the growth rate of mites on dry cured hams was reduced when the relative humidity was decreased below 60% (Garcia, 2004). Therefore, manipulating the environmental conditions of dry cured ham aging facilities may contribute to the control of *T. putrescentiae* on dry cured ham in conjunction with the application of food grade ingredient infused nets.

The objectives of this research were: 1) to determine the most effective temperature and relative humidity combinations to inhibit mite reproduction and development during the aging of dry cured hams in untreated and food grade ingredient infused nets; and 2) to assess and identify the types of fungal species that grow on dry cured hams at the different temperature and RH combinations.

CHAPTER II

LITERATURE REVIEW

2.1. Dry Cured Ham

Prior to refrigeration, dry curing was a commonly used method to preserve meat products such as pork hams. The traditional dry curing of hams was adapted from meat preserving techniques of the southern European Mediterranean culture and is currently effective at industrially producing specialty dry cured ham products (Toldrá, 2002). Dry cured hams are predominantly produced in countries that experiences mild winters, and hot humid summers such as some European countries, China, and the southeastern United States (Toldrá and Aristoy, 2010). Dry cured hams differ due to the breed, diet, weight and age of pigs, and processing conditions (Petrova et al., 2015a). Popular types of hams include Spanish Iberian, Chinese Jinhua, and American country ham (Toldrá and Aristoy, 2010).

American dry cured hams are uncooked, cured, dried, smoked or unsmoked meat products that are produced from the hind leg of a pork carcass (USDA FSIS 9 CFR 319.106). The distinctive salty and meaty flavor and firm texture of dry cured hams are developed during a curing and aging process that consists of the application of salt, nitrate and/or nitrite to the surface of the hams (USDA FSIS 9 CFR 319.106). Dry cured hams are aged for 3 to 24 months and require certain environmental conditions (65-75%

relative humidity (RH) and 20-30°C) to acquire the desired aroma, flavor, and textural attributes (Marriott et al., 1992).

2.2. Dry Cured Ham Processing

The traditional technology for manufacturing dry cured hams mainly involves curing and dry-aging. Fresh 7-14 kg hams that are obtained from 6 month old hogs are cured using a mixture of salt, nitrate and/or nitrite, sugar, and seasonings (Ockerman et al., 2002). The curing mixture is applied directly to the ham's surface and remains on the hams for approximately 14-28 d at 2-7°C and 75-90% RH (USDA FSIS 9 CFR 318.10). During the curing phase, salt penetrates the surface of the hams, which results in moisture removal due to osmotic dehydration. Salt equilibration is conducted after curing at approximately 13°C for approximately 2 weeks and supports partial water removal, reduces energy costs, and inhibits the development of spoilage microorganisms (Ockerman et al., 2002; Rentfrow et al., 2012; Petrova et al., 2015a).

Dry-aging contributes to dry cured hams' distinct aroma, flavor, and texture (Rentfrow et al., 2012). During this stage of production, cured hams are commonly hung on racks via hooks with or without ham nets and are stored at 25 to 30°C and a relative humidity of 65-75% (Marriott et al., 1992). At these conditions, water is removed from the cured hams by evaporation, and these conditions are maintained until the ham loses at least 18% of its original weight and contains a minimum of 4% salt (USDA FSIS 9 CFR 319.106). Dry cured hams are aged from 3 to 24 months for the development of the highest quality hams. Enzymatic degradation occurs as aging time increases, which contributes the unique flavor and texture of dry cured ham (Petrova et al., 2015b).

2.3. Pest Infestations of Dry Cured Hams

Dry cured ham's fat and protein content contributes to its susceptibility to several pests including red-legged ham beetles (*Necrobia rufipes*), cheese skippers (*Piophila casei*), ham mites (*Tyrophagus putrescentiae*) and fungi (*Penicillium nordicum*) (Gulati and Mathur, 1995; Zhao et al., 2016a). Several of these species infest stored food products including cheeses, dried grains, pet foods, and dry cured hams (Rentfrow et al., 2008; Zhao et al., 2016b). The pest that is most difficult to control is *T. putrescentiae* (Shrank), also known as the ham, mold or cheese mite. This is mainly due to their size, body structure, dispersion, rapid reproduction and development, and their use of fungal species as a food source. The ham mite has a small, translucent body structure that ranges from 280-420 μm in length. Adult mites have two body regions (gnathosoma and idiosoma) that are fused together. The gnathosoma consists of the mouth and specialized feeding appendages and the idiosoma consists of legs that support sensory recognition, reproduction, and locomotion (Edde et al., 2012; Zhang et al., 2017)

Ham mites commonly infest dry cured hams via rapid dispersion across the ham surfaces. Their ability to penetrate ham surfaces and enter into the cracks and crevices of the cured hams causes product and economic losses (Žďárková, 1971; Zhao et al., 2015). The rate of dispersion tends to increase under unfavorable conditions such as product degradation, overcrowding, and/or the lack of nutrients, which leads to mite movement to other food sources and equipment, potentially causing infestations in new locations (Kuwahara et al., 1975; Edde et al., 2012; Zhang et al., 2017).

Mite reproduction and development rate is influenced by population, health, food source, relative humidity, and temperature (Collins, 2012; Vacante, 2016). *T. putrescentiae* generation time is 8 to 21 d at 60-80% RH and temperatures ranging between 20-32°C (Mueller et al., 2006). Each female mite can lay approximately 500 eggs throughout her lifespan at optimal rearing temperatures, which is between 22 and 26°C (Graham et al., 2012). Each egg develops into an adult mite in approximately 9-12 days at 20°C and 85% RH (Boczek, 1991).

The longevity of the ham mites is highly dependent on environmental conditions. The lifespan of *T. putrescentiae* decreases as the temperature increases, and their lifespan is directly proportional to relative humidity (Sánchez-Ramos and Castañera, 2005; Mueller et al., 2006; Zhang et al., 2017). At a temperature of 9°C, a mite can live for approximately 115 days in comparison to 43 days at 31°C. Researchers have reported that *T. putrescentiae* mites have a shorter lifespan at RHs greater than 85%, which is influenced by factors such as the sex of the mite as well as food source available. For example, *T. putrescentiae* mites can live without food for at least 2 months at room temperature. In addition, male mites tend to live longer than female mites on a food source such as wheat germ (Wu et al., 1974; Boczek, 1991).

Dry cured ham aging conditions provide an environment that is conducive for fungal growth. Various fungal species of *Aspergillus*, *Eurotium*, and *Penicillium* genera grow on the surface of dry cured ham, which contributes to its rich meaty flavor and aroma (Wu et al., 1974). Although some fungal growth is favorable in dry cured ham processing, some fungi support mite infestations. Fungi and mites benefit from the

presence of each other on dry cured hams. As mites feed on fungi and rapidly disperse, they carry fungal spores to new sources of food, which contributes to the growth and development of the fungi (Hubert et al., 2004; Zhang et al., 2017).

2.4. Methods of Pest Control

Several mite management strategies have been developed to combat the challenge of controlling pests with chemical pesticides. These methods include, but are not limited to chemical, biological, physical methods for the control of ham mites. For example, the enhancement of facility sanitation programs, the development of ham mite traps, food grade coatings and food grade coatings infused nets have been researched in effort to control mite infestations in the dry cured ham industry (Amoah, 2016). Temperature and RH have been mentioned by processors as a means to control mites, but no research has been reported on the effects on mite growth on dry cured hams. These potential alternatives could potentially be included in an Integrated Pest Management (IPM) program for dry cured ham processing in an effort to monitor and detect *T. putrescentiae* mites on dry cured hams, control mite infestations, and ultimately avoid *T. putrescentiae* mite infestations (Amoah, 2016; Zhang et al., 2017).

2.4.1. Methyl Bromide

The most commonly used method to control ham mite infestations in the United States is fumigation with methyl bromide (MB). This fumigant is an odorless, colorless gas that was commonly used in multiple industries to control various pests, including *T. putrescentiae* mites. Conversely, methyl bromide is listed as a stratospheric ozone layer depleting substance in the Montreal Protocol (Fields and White, 2002). This is an

international agreement that was signed by 182 countries to phase out the use of MB by 2015 (UNEP, 1992). The phase out process of MB was designed to gradually reduce the use of MB from 25 to 100% by 2005, with the exception of critical use exemptions (CUEs) (Bell et al., 1996; Osteen, 2003). Only annual requests for commodities such as figs, dried plums, and dates were exempt due to their need for rapid fumigation to meet their marketing seasons (EPA, 2013). The dry cured ham industry was awarded CUEs from 2005 to 2016 since no other effective alternatives were registered to control red-legged beetles, dermestid beetles, ham skippers, ham mites, and other pests (Johnson et al., 2012; EPA, 2013). Currently, the American dry cured ham industry has been granted access to the remaining MB stocks to help control infestations in dry cured ham processing facilities (EPA, 2017b). The limited supply of MB has led to a 10 fold increase in cost from 2000 to 2015 and made it difficult for small processors to afford MB fumigation (EPA, 2017a). Therefore, various researchers from universities and companies are pursuing alternative means of fumigation to control mite infestations.

2.4.2. Alternative Fumigants

Potential fumigant alternatives to methyl bromide, including phosphine, sulfuryl fluoride, carbon dioxide, and ozone have been evaluated in benchtop experiments for their effectiveness at controlling *T. putrescentiae* and red-legged beetle infestations (Sekhon et al., 2010a). Phosphine is an effective and low cost fumigant that is toxic to aerobically respiring organisms such as *T. putrescentiae* (Nath et al., 2011; Zhao et al., 2015). When applied at 1000 ppm for 48 h, phosphine achieved 100% mortality of all life stages of *T. putrescentiae* in a controlled environment that was maintained at 20°C. In

addition, phosphine fumigation did not affect sensory characteristics of the ham (Sekhon et al., 2010a). Several fumigation trials in various environments under different conditions: one under lab conditions, one in simulated aging houses, and one in a commercial processing plant, were conducted by Zhao et al. (2015) to evaluate pest mortality, phosphine residue, and sensory effects due to fumigation. It was concluded that phosphine was effective at controlling *T. putrescentiae* mites under laboratory conditions. However, phosphine was ineffective in a commercial setting, largely due to the corrosion and damage of metal parts and copper wiring in processing facilities.

Sulfuryl fluoride is an alternative to methyl bromide in some industrial applications and was registered in 2005 for use in the dry cured ham industry (EPA, 2005). Sekhon et al. (2010b) reported that sulfuryl fluoride was effective at controlling all life stages of red-legged beetles at 36 mg/L. Conversely, greater concentrations of sulfuryl fluoride was required, three times maximum label rate of sulfuryl fluoride, to achieve 95% mortality of the *T. putrescentiae* in laboratory studies. Also, residual sulfuryl fluoride and fluoride ion concentrations were directly proportional to sulfuryl fluoride fumigation concentration, which led to sulfuryl fluoride and fluoride ions exceeding the limits of 20 ppm fluoride ion and 0.01 ppm sulfuryl fluoride in dry cured hams (EPA, 2005; Sekhon et al., 2010b). Therefore, sulfuryl fluoride was concluded as an ineffective alternative to methyl bromide for the control of *T. putrescentiae*.

Carbon dioxide was evaluated for its effectiveness at controlling *T. putrescentiae* infestations (Hasan et al., 2016). After 144 h of exposure to 80% carbon dioxide, 95-100% mortality of all life stages of red legged ham beetles and mites were achieved

(Sekhon et al., 2010c; Hasan et al., 2016). However, it is impractical to utilize carbon dioxide since dry cured ham facilities are not air-tight and economic losses would occur due to the lengthy exposure time needed to kill *T. putrescentiae* mites.

Ozone was also evaluated under laboratory conditions and applied at 175 ppm for 48 hrs. This study rendered 100% mortality for all life stages of *T. putrescentiae* mites and red-legged beetles. However, ozone's lack of ability to penetrate ham surfaces reduces its effectiveness on *T. putrescentiae* mites that can hide in the cracks and crevices of dry cured hams (Sekhon et al., 2010c; Hasan et al., 2016). In addition, ham producers have evaluated ozone in processing plants for its efficacy at controlling mites and have reported that it was ineffective at controlling mite infestations (Edwards, 2016).

2.4.3. Food-grade Ingredient Coatings

The application of food grade coating on dry cured ham originated from the idea of using coating agents as a carrier of food grade ingredients that are effective at controlling mite infestations. Abbar et al. (2012) tested several food grade ingredients including vegetable and animal oils, propionic and citric acids, short-chain alcohols, and butylated phenol preservatives to evaluate *T. putrescentiae* reproduction and development after dipping ham cubes in these solutions and incubating for two weeks. Results indicated that 50 or 100% propylene glycol, and 100% lard were effective at controlling mite growth under laboratory conditions (Abbar et al., 2012). These findings motivated the development of food grade coatings including polysaccharides, lard, and/or propylene glycol that were evaluated for their efficacy at controlling mite infestations on dry cured hams under laboratory conditions.

Zhao et al. (2016) developed various food grade coating formulations that consisted of polysaccharides, including xanthan gum, carrageenan, and propylene glycol alginate. There was a 50% reduction in mite number on ham cubes in comparison to control cubes when xanthan gum (XG, 1%) and a combination of propylene glycol alginate (PGA, 1%) and carrageenan (CG, 1%) were used. Various levels of propylene glycol (PG) were tested at room temperature and 70% RH, and mite growth was completely inhibited with either zero to 2 mites present on ham cubes when XG was combined with at least 20% PG and PGA+CG was combined with at least 10% PG, respectively. Treatments that contained 20 to 50% PG were evaluated and rendered complete inhibition on mite reproduction (Zhao et al., 2016b).

Mite reproduction assays were conducted by inoculating 900 mites per whole hams coated with PGA+CG+20% or 40% PG. After 6 weeks of aging, fewer than 10 mites were present on the hams (Abbar et al., 2016a). *T. putrescentiae* mite's reproduction was drastically reduced from the original inoculum level, indicating that these food grade coatings could potentially be an effective alternative for methyl bromide. Since dipping and coating requires additional processing steps, it was determined that it would be more economically feasible to infuse the food grade coating technology into ham nets that are used to hang the dry cured hams during aging.

2.4.4. Food-grade Ingredient Infused Nets

Further research has adapted textile technologies via the infusion of the food grade coatings into small mesh netting material, which dry-cured hams commonly hang in while aging, to control mite reproduction (Campbell et al., 2016b). Polyester and/or

cotton blend nets infused with the food grade coatings were used during whole ham aging at 25°C and 65% RH. These researchers reported that polyester/cotton blend or cotton nets treated with PG delivered by XG or CG + PGA were effective at controlling mite growth on ham cubes at concentrations as low as 10% PG. The use of XG + 10% PG and XG + 20% PG reduced the mite count when compared to the control ham cubes. CG + PGA + 10% PG in cotton and blend nets was effective at inhibiting mite growth by keeping the numbers as low as 31 and 62, respectively. All treatments were effective at controlling mite reproduction without detectable changes in the sensory properties and quality of hams (Campbell et al., 2016b).

Coatings may dry out when embedded in netting material due to exposure to environmental changes, which may reduce their effectiveness at controlling mite infestations over time. However, time dependent studies performed by Zhang et al. (2017) indicated that the XG+PG and CG+PGA+PG coatings remained effective at controlling mites for at least 10 weeks under laboratory conditions. In this study, the treatments included no net on the cube, untreated nets, and nets treated with gums and PG. Results indicated that regardless of the netting duration prior to mite inoculation, the number of *T. putrescentiae* produced in the assays was less on ham cubes in treated nets after two weeks of culture when compared to any of the treatments lacking PG. In addition, XG + PG treated samples had fewer mites than ham cubes without nets or with untreated nets and no differences observed between XG and CG + PGA treated nets of the same concentration of PG (Zhang et al., 2017). Zhang et al. (2017) also reported the presence of mold on XG + PG and CG + PGA + PG nets with low levels of PG.

Therefore, it was beneficial to increase the concentration of PG in the food grade ingredient infused nets to effectively control fungal growth and *T. putrescentiae* infestations.

2.4.5. Temperature

Environmental temperature is a practical physical method for the control of mite infestations on various products. *T. putrescentiae* mites are able to tolerate low and high temperatures ranging between, 7-10°C and 35-38°C, respectively. However, their optimal temperature range for maximum growth and development is 25 to 33°C (Fields, 1992; Hubert et al., 2010; Collins, 2012; Zhang et al., 2017). Management of *T. putrescentiae* via temperature control is influenced by various factors including ham mite's developmental stage, time of exposure, acclimation, and relative humidity (Barker, 1967; Sánchez-Ramos and Castañera, 2001).

Egg stage mortality at 11.2°C was 67%. Thirty-seven percent mortality was achieved for immature mites at the same temperature (Barker, 1967). However, recent studies indicated 100% mortality for both eggs and immature stages of mites, with eggs being more tolerant of low temperatures (-20 to -5 °C) than immature mites (Abbar et al., 2016b). Complete mortality of ham mites can be achieved at temperature and time combinations between -5°C for 48 h to -20°C for 12 h, respectively. Maintaining mites at low temperatures such as -18°C for 5 h has achieved 90% mortality of *T. putrescentiae* at all life stages (Eaton and Kells, 2011), but operating at these conditions are not economical for the manufacturing of dry cured hams since it would require the introduction of large aging rooms with the capability to function as freezers.

Barker (1967) noted that rapid development in both eggs and immature mites were seen between 22.2 and 32.2°C, with a reduction in development occurring at and beyond 36.7°C. Recent studies stated that 100% mortality of ham mites can be achieved at 40 to 45°C when evaluated at 90% RH (Abbar et al., 2016b). Temperatures above 40°C stimulate the desiccation which leads to increased mite mortality of *T. putrescentiae* mites alone and increases the rate of dehydration of dry cured hams. The loss of body fluids through the flexible body surface of a *T. putrescentiae* mite increases as the body surface becomes brittle due to sclerotized cuticles when exposed to temperatures greater than 40°C (Wharton et al., 1979). The mite's nervous system is sensitive to high temperatures that destabilize their phospholipid membranes, which results in death (Fields, 1992).

Processing dry cured hams at higher temperatures (40°C) also affects the finished product quality (Abbar et al., 2016b; Zhang et al., 2017). As the ham dehydration rate increases, cracks form in the lean muscle of the pork hind leg (Sanchez-Molinero et al., 2010). It is also regulated that the internal temperature of dry cured hams should not exceed 35°C during the drying and smoking phases to avoid the inactivation of enzymes needed for the development of dry cured ham sensory characteristics (USDA FSIS 9 CFR 319.106). Although high temperatures are effective at preventing mite infestations in stored food products, mite's ability to adapt to higher temperatures increases mite's temperature threshold of survivability and must be thoroughly examined to ensure that mite infestations are controlled (Fields, 1992; Zhang et al., 2017).

2.4.6. Relative Humidity

Relative humidity is a crucial parameter for aging dry cured hams. Minute changes can alter the dehydration rate of the hams (Sánchez-Ramos et al., 2007). Dry cured hams are typically aged at relative humidities of 65 to 75% RH for 3 to 24 months. This provides optimal conditions for the growth and development of *T. putrescentiae* (Marriott et al., 1992). *T. putrescentiae* are also able to survive at relative humidity values below 55%, due to their ability to adapt to their surroundings (Arlian, 1992; Sánchez-Ramos et al., 2007).

Since temperature is a limiting function of relative humidity, low values of relative humidity cause fluid loss from the body structures of ham mites, but the body fluids that are lost are compensated for via the mites extracting water vapor from the environment (Arlian, 1992). The supracoxal gland of ham mites is responsible for capturing moisture from the air and maintaining the water balance that is necessary for survival (Wharton and Furumizo, 1977; Arlian, 1992). Since this gland can lose it functionality via changes in relative humidity, it is beneficial to determine the effect of environmental moisture on the growth and development of *T. putrescentiae* on dry cured hams.

Previous studies have shown that *T. putrescentiae* prefer higher humidity (85-95%), but their ability to adapt to their environment promotes their survival at relative humidity values below 65% (Kheradmand et al., 2007; Sánchez-Ramos and Castañera, 2007). Cutcher (1973) reported that mites adapt to low humidity environments by huddling together to reduce the surface area and water vapor stress, finding shelter in an

environment with a substantial food source, and/or moving to locations that acts as a barrier against water evaporation. In particular, dry cured hams commonly acquire cracks and crevices in the lean muscle of the ham when aged at low values of RHs under laboratory conditions. The low humidity environment forced mites to cluster together in the cracks of the hams to survive (Cutcher, 1973; Sánchez-Ramos and Castañera, 2005).

Relative humidity values (65-85%) commonly vary throughout the aging process with alterations in temperature and affect the moisture content and water activity of dry cured hams. The effect of relative humidity of the drying air among other parameters including moisture content and water activity were evaluated (Arnau and Guerrero, 1994). The experiments were conducted for 40 days at 4°C and at 52, 78 and 85% RH. The study showed that the highest moisture content as well as the highest water activity was observed in the samples at 85% RH, and the lowest values were in the samples at 52% RH. These same trends were confirmed in studies that were tested at three different relative humidity values: 70–75, 75–80 and 80–85% at 4°C for the impact on A_w (Gou et al., 2003). Hams that were aged at lower relative humidity had a decreased moisture content and water activity. However, aging hams in low relative humidity conditions can lead to poor product quality via cracks and crustiness on the ham surface (Arnau et al., 2003). Therefore, further research is needed to determine the effects of environmental conditions on mite infestations for inclusion in integrated pest management programs.

2.5. Fungal Contamination on Dry Cured Hams

2.5.1. Mold

Environmental conditions during the production of dry cured hams facilitates both desired and undesired mold growth on the surface of the hams (Rodrıguez et al., 1998). The growth of some molds is considered desirable due to their ability to enhance aroma, flavor, and texture characteristics of the dry cured meat product, while undesired mold growth can cause product spoilage as well as produce mycotoxins at temperatures greater than 20°C that are harmful to humans (Sutic et al., 1972). The dry cured ham surface's mycoflora consists of species in the *Aspergillus*, *Eurotium*, and *Penicillium* genera (Wu et al., 1974; Asefa et al., 2010).

Dry cured hams are usually aged at 65-75% RH and temperatures greater than 28°C, which is a conducive environment for fungal growth to occur on dry cured ham surfaces. In one study, *Aspergillus*, *Eurotium*, and *Penicillium* species represented more than 97% of fungal isolates on dry cured hams (Campell-Platt, 1995; Comi et al., 2004). *Aspergillus flavus*, *A. repens*, and *A. ochraceus* are several of the strains of mold found on ham surfaces during the aging phase of processing (Comi et al., 2004), with the *Aspergillus spp*. as the most common and dominant mold on dry cured hams (Rojas et al., 1991). These fungi could potentially produce aflatoxins on dry cured ham surfaces, and studies via artificial inoculation of ham pieces indicated that aflatoxins are commonly produced at temperatures greater than 15°C and at water activities greater than 0.90 on experimental substrates. However, there is not definite evidence that hams contain

harmful amounts of aflatoxins and need to be further investigated (Rojas et al., 1991; Peromingo et al., 2016).

Eurotium species are able to grow at relative humidity values less than 80% (Rodrıguez et al., 1998; Asefa et al., 2009). At a RH less than 80%, *E. repens* strains made up 66% of the surface mold and were responsible for the unique aroma of dry cured hams. The presence of *Eurotium* species indicates that the ham has ripened and that the ham has reached the desired water activity of 0.88-0.79 (Rodrıguez et al., 1998). *Pennicillium frequentans*, *P. verrucosum*, and *P. expansum* are frequently present on dry cured ham surfaces and are dominant molds on food products with water activity between 0.85 and 0.90 (Pitt and Hocking, 2009). *Pennicillium spp.* can also develop at relative humidity values greater than 60% on products such as hams, but they are not desired because they can produce toxic metabolites, off-flavors, and off odors (Northolt and Bullerman, 1982; Comi et al., 2004). Various fungal species can attract or repel *T. putrescentiae* mites; however, studies have revealed that ham mites prefer both *Pennicillium* and *Eurotium* species, but avoid *Aspergillus spp.* (Hubert et al., 2003).

2.5.2. Yeast

T. putrescentiae feeds on different fungi including various species of yeast that are present on dry cured ham surfaces. Yeast appears as a "white powder" film on dry cured ham with water activities of 0.85-0.92 and temperatures between 10 and 28°C (Deak and Beuchat, 1996). The most predominant species of yeast that are found on hams include *Debaryomyces hansenii*, *Torulopsis candida*, and *Torulopsis famata*, but *D. hansenii* is the most dominant yeast species after aging. Rodriguez (1998) reported that

yeast contribute to dry cured hams' sensory qualities via their lipolytic and proteolytic activity that contributes to dry cured ham's distinctive flavor and texture.

CHAPTER III

MATERIALS AND METHODS

3.1. Food-grade Coating Materials

Xanthan gum (Pre-hydrated Ticaxan® Rapid-3 powder, TIC Gums, Belcamp, MD 21017), carrageenan (Ticagel® 795 Powder, TIC Gums), propylene glycol alginate (Tica-algin®, PGA, TIC Gums), and propylene glycol (Essential Depot, Sebring, FL) were used to formulate coatings. The coatings were infused into ham stockinettes (25 cm wide) that were comprised of 50% cotton and 50% polyester with a stitch density of 112 loops/cm^2 (Ennio Meat Packaging Specialists, Smoke Nets, Aurora, IL, 60504).

3.2. Food-grade Coating Net Infusion

Xanthan gum (XG), a cold water-soluble polysaccharide, was dissolved in tap water at room temperature (25°C) prior to mixing with propylene glycol (PG). Hot water-soluble gums (e.g. carrageenan (CG) and propylene glycol alginate (PGA)) were mixed with constant stirring with PG and then added to water while heating to a final temperature of 90°C until the liquid was clear. The solution was then cooled to 65°C before infusing it into the nets.

Ham nets were dipped into one of the two different food grade coating treatments: 1) 1% XG and 40% PG or 2) 1% CG, 1% PGA, and 40% PG. The two treatments were individually infused into the specified net using a netting machine (Midwest Metalcraft &

Equipment Company, MI 65360) that pressed the coated nets between two rollers to minimize the amount of coating used. The pressed, coated nets were weighed, and the coating weight per meter of nets was calculated to determine the amount of gum absorbed into the fibers of the nets. The 1% XG and 40% PG nets absorbed 170 ± 5 g/m and the 1% CG, 1% PGA, and 40% PG absorbed 184 ± 5 g/m. The infused nets were vacuum-packaged in vacuum pack bags (40 cm × 60 cm, 3 mil. standard barrier, nylon/PE Clarity Vacuum Pouches; Kansas City, MO) using a dual-chamber vacuum packaging machine (Model 2100, Koch Equipment LL., Kansas City, MO) and stored at 25°C until use.

3.3. Ham Cubes and Slices Preparation

Dry-cured hams that weighed 6-8 kg and were aged for 3-6 months were purchased from commercial ham plants. The hams were transversely cut into 2.5 cm slices using a band-saw (B16-P, Butcher Boy- Lasar MFG Company, Los Angeles, CA, 90023). Slices were individually vacuum-packaged in vacuum pack bags (40 cm × 60 cm, 3 mil. standard barrier, nylon/PE Clarity Vacuum Pouches; Kansas City, MO) and stored at 4°C until used.

To prepare ham cubes for the mite infestation study, three 2.5 cm thick slices were used. One cube (2.5 × 2.5 × 2.5 cm^3) was selected from three different muscle groups of each slice: one from the *adductor* and *semimembranosus* muscles, one from the *semitendinosus* muscle, and one cube taken from the *biceps femoris* muscle. These three cubes were randomly assigned to and wrapped in an untreated or treated net and tested at each temperature (24, 28, and 32°C) and RH (55, 65, 75, and 85%) combination.

Nine different 2.5 cm thick slices were randomly selected from the three different hams (three slices per whole ham). Smaller slices (2.5 cm × 9.0 cm × 15.5 cm) were cut from the original ham slices at 2.5 cm above the bone so that the slices would fit inside the container in which it was inoculated with mites. The smaller slices consisted of the following muscle groups: *vastus intermedius*, *vastus lateralis*, *vastus medialis*, *pectineus*, *adductor*, *biceps femoris, semitendinosus, and semimembranosus* muscles. Each slice was assigned to and wrapped in an untreated or treated net and tested at each temperature and RH combination.

3.4. Rearing Mite Cultures

Tyrophagus putrescentiae were cultured via methodologies stated in Abbar et al. (2016a). *T. putrescentiae* stocks were reared biweekly at Mississippi State University in ventilated 355 mL glass jars (Wide-mouth jar, Ball Mason, Ball Corp., Broomfield, CO) that were filled with fresh mite diet (Abbar et al., 2016a). The ventilated rearing jars were contained in a basin that was filled with soap and water at its base and petroleum jelly smeared on the edges. The basins were stored in a locked storage cabinet and the mite cultures were maintained at 23 ± 2°C and 80 ± 5% RH.

3.5. Mite Infestation Assays

Each individual ham cube (2.5 × 2.5 × 2.5 cm^3) was placed in a ventilated glass jar (216 mL, 65 mm diameter, 55 mm height; Ball Corp., Broomfield, CO) and labeled with a random three-digit code. Black construction paper (5 cm diameter) covered the bottom of each of the glass jars and filter paper (Whatman No. 1, 90 mm diameter; GE Healthcare UK Limited, Amersham, UK) covered the top that was sealed by a jar ring.

Each ham cube was inoculated with twenty mixed sex, adult ham mites inside the jar before sealing with the filter paper and jar ring. The jars were placed in a soap and water bath, and the edges of the container were covered with petroleum jelly to prevent mites from escaping.

The jars that contained inoculated ham cubes were then placed in a 0.28 cubic meter pesticide treated environmental control chamber (LH-10 Economy Line Humidity Chamber, Associated Environmental Systems, Acton, MA), and the treated samples were stored for 14 d at temperatures and relative humidity combinations of 24, 28, and 32°C and 55, 65, 75, and 85% RH. Each temperature and relative humidity combination was tested in random order. After 14 d of incubation, the ham cube, jar, black construction paper, filter paper, and netting were separated and individually placed in a petri dish. Each item was evaluated for mite infestation by counting the number of living mites on each component under a microscope (Model 568, American Optical Company, Buffalo, NY).

The ham slices were tested similarly to the ham cubes. The 2.5 cm × 9.0 cm × 15.5 cm slices were assigned to and wrapped in untreated or treated nets. To simulate a ham aging house, each wrapped ham was hung using a 1.5 mm silver coated copper wire (Cousin Corporation of America, Largo, FL) in a 3.8 L bottle (Paksh Novelty Wide Mouth Clear Glass Jar plus Metal Lid with Airtight Liner Seal, Garden Plus) that was labeled with a three-digit code. Black construction paper (14 cm diameter) covered the bottom of the glass jars, and filter paper (Whatman No. 40., 110 mm diameter; GE Healthcare UK Limited, Amersham, UK) was placed over the mouth of the jar and sealed

with a jar ring after the slices were inoculated with 50 mites and sealed tightly with petroleum jelly around the lid. The containers were then placed in a basin of soapy water trimmed in petroleum jelly, and the jars in the water basins were stored for 14 d in the environmental chamber along with ham cubes. Living mites were observed after 14 d of incubation at each temperature and relative humidity combination. Due to the large surface area of the ham slice, mite reproduction on ham slices was observed under a microscope using a flashlight and a magnifying glass.

3.6. Water Activity and Moisture Content

Water activity and moisture content was measured for each sample before and after the 14 d incubation period in the environmental chamber. Prior to inoculation, randomly selected pieces from each muscle groups described in section 3.3 were used to measure the water activity (AquaLab Series 3 TE, Decagon Devices, Inc., Pullman, WA) for ham cubes and slices. A 0.16 cm × 2.5 cm × 5 cm cut from the surface of the *adductor* and *semimembranosus* muscles was used to measure the water activity and moisture content of each ham slice after 14 d of incubation in an environmental control chamber. To determine the moisture of each sample, 2.0 ± 0.1 g of minced ham was dried in an oven at 100 ± 2°C until a constant weight was obtained (AOAC, 1998). The moisture content (% wet basis) was calculated via the following equation:

$$\%\ Moisture = \frac{Initial\ ham\ wieght\ (g) - Dried\ ham\ wieght(\ g)}{Dried\ \ ham\ wieght\ (g)} * 100 \qquad (1)$$

3.7. Evaluation of Mold Growth

The amount of mold present on the ham slice surfaces was evaluated visually by a trained panel (n=6) after 14 d of incubation. Mold growth was quantified using a 0 to 100 percentage scale, where 0 was no visible mold and 100 indicated that visible mold covered the entire ham slice. When mold was present on the large ham samples, the mold was aseptically cultured on potato dextrose agar (PDA, CM0139, Oxoid LTD, Basingstoke-Hampshire, England) using a sterilized inoculation needle.

3.8. Mycological Assessment

3.8.1. Mold and Yeast Isolation

Mold samples were isolated by taking a portion of the aerial mycelium with a sterile inoculation needle, and each sample was aseptically inoculated at the center of a solidified PDA that was contained in a sterilized petri dish (Fisherbrand, Sterile 95 mm × 15 mm Polystrene Petri Dish, Waltham, MA 02451). The inoculated mold plates were incubated at 25 ± 2°C for 5 to 7 d prior to inspection. Yeast samples, which appeared as a slimy film and/or defined globular colonies on ham slices, were collected and aseptically streaked on PDA with a sterilized 1µm inoculating loop. The yeast samples were incubated for 3 d at 25 ± 2°C.

3.8.2. Mold and Yeast Identification

Isolates were identified at the genus level for mold and species level for yeast using phenotypic methods. A molecular method was also used to identify fungal growth on the dry cured hams 14 d after treatment, which identified mold and yeast isolates at the species level.

3.8.2.1. Traditional Methods

Mold and yeast samples were sub-cultured on PDA plates using the agar block slide technique that is described in *Laboratory Methods in Food Microbiology* (Harrigan, 1998). After sterilization of all materials, forceps were used to aseptically place a sheet of filter paper (Whatman No. 1, 90 mm diameter; GE Healthcare UK Limited, Amersham, UK) in a petri dish. Next, 1.5 mL of sterile deionized water was used to saturate the filter paper, and a v-shaped rod (Fisherbrand L-shaped cell spreader, 5 cm, Waltham, MA 02451) that supported a microscope slide was placed on top of the filter paper.

A 1 cm^2 square block from a previous solidified PDA plate was aseptically cut, removed, and placed in the center of the microscope slide. Using one mold sample per block medium, the four cut surfaces of each block medium were inoculated with mold using an inoculation needle, while one isolated yeast colony was streaked on the top of the 1 cm^2 block. A sterilized coverslip was then place on top of the inoculated agar surface, and the petri dish was covered with a lid and wrapped in parafilm. All mold samples were incubated for 5 d at 25°C and yeast samples were incubated for 3 d at 25°C.

During incubation, the mold and yeast colonies adhered to the coverslip. Wet mounts were prepared by carefully removing the coverslip from the agar block. The coverslip was aseptically mounted in lactophenol-cotton blue (Difco Laboratories, Detroit, MI 48201) on a clean microscope slide, and the morphological characteristics were observed at both macroscopic and microscopic views.

3.8.2.2. Molecular Methods

Genomic DNA extraction, amplification, and sequencing were used to verify the most prevalent mold and yeast isolates from the surface of the ham slices (Asefa et al., 2010). Mold samples were taken directly from a 7 d incubated PDA plate, frozen in liquid nitrogen, and then grounded to fine powder with a mortar and a pestle. Each powdered sample was transferred to a 1.5 mL Eppendorf tube and homogenized in 700 µL of cetyltrimethylammonium bromide (CTAB) DNA extraction buffer. A 4 mm loopful from each pure yeast sample was transferred to a 1.5 mL Eppendorf tube that contained 100 µL of an extraction buffer 200 mM lithium acetate (Sigma, St. Louis, MO 63178) in 1% sodium dodecyl sulfate (Fisher Scientific, Fair Lawn, NJ 07410)) and vortexed for 30 sec. An additional 100 µL of extraction buffer was added to each yeast sample, and the sample was then vigorously vortexed. The yeast samples were then frozen in liquid nitrogen for 90 sec and immediately thawed in a 65°C water bath for 90 sec. The freezing and thawing method was repeated 3 times. Samples were vigorously mixed using a vortex between each repetition of freezing and thawing to help break down the cellular structure of the yeast. After freezing and thawing, an additional 500 µL of the LA-SDA extracting buffer was added to the yeast samples. Both mold and yeast DNA samples were incubated in a water bath at 65°C for 20 min, with samples inverted every 5 min (Murray and Thompson, 1980).

For each mold and yeast sample, 5 µL of RNase A (10 mg/ml) was added to the sample solution prior to incubation at 37°C for 15 min. Samples were then suspended in 700 µL of phenol : chloroform : isoamyl alcohol (25 : 24 : 1, v/v/v). After centrifuging

the samples at 12,000 × *g* for 10 min at 20°C, extraction was repeated by transferring the upper aqueous solution into 700 µL of phenol : chloroform : isoamyl alcohol (25 : 24 : 1, v/v/v). The DNA of both mold and yeast was further extracted with 700 µL of chloroform : isoamyl alcohol (24:1, v/v) and centrifuged at 14,000 × *g* for 5 min at 20°C,. The upper aqueous solutions, that contained the DNA, was precipitated by adding 3 M 10% sodium acetate (1:10, v/v and pH 4.8) and 100% ethanol (1: 2.5, v/v), and storing the samples at -20°C for 16-20 h.

The DNA samples were centrifuged at 14,000 × *g* for 15 min at 4°C, and the aqueous solution was removed. The DNA pellets were washed twice: once with 500 µL of 75% (v/v) ethanol and once with 150 µL of 75% (v/v) ethanol. The DNA samples were centrifuged again at 14,000 × *g* for 5 min at 4°C, and the ethanol rinse was removed. The pellets were then air-dried for 15 min at 25°C. The dried DNA samples were then dissolved in 20 µL of Monarch DNA Elution buffer (Monarch, Ispwich, MA 01938), centrifuged, and stored at -20°C until use.

A NanoDrop spectrophotometer (ND-2000, NanoDrop Technologies, Wilmington, DE) was used to determine the concentration of DNA in each sample. The quality of the DNA samples was evaluated on a 1% (w/v) agarose gel composed of 20 mM Tris-HCl (pH 7.5), 7.5 mM sodium acetate, 0.5 mM EDTA, and 0.01% SYBR safe (SYBRbSAFE in DMSO, S33102, Invitrogen, Carlsbad, CA 92008). Gel electrophoresis was conducted at 100 V for 20 min in a buffer consisting of 20 mM Tris-HCl (pH 7.5), 7.5 mM sodium acetate, and 0.5 mM EDTA. Gel images were captured with a ChemiDoc

XRS+ system that was equipped with version 5.0 of Image Lab software (Bio-Rad Laboratories, Hercules, CA).

3.8.2.2.1. DNA Amplification via the PCR Technique

The mold's genomic DNA (gDNA) was processed via PCR (Polymerase Chain Reaction) techniques using fungus-specific universal primers, ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-GCATATCAATAAGCGGAGGA-3') (White et al., 1990). The amplification of D1/D2 region of yeast's rDNA was performed via PCR techniques using the forward primer ITS38 (5'-GCATCGATGAAGAACGCAGC-3') and the reverse primer NL-4 (5'-GGTCCGTGTTTCAAGACGG-3'). For each mold and yeast sample, 2.5 µL of gDNA (200 ng/µL) and 1.25 µL each of the specified forward and reverse primers were mixed with a PCR reaction mixture that was composed of the following reagents: 10 µL of 5X Colorless GoTaq Reaction Buffer, 1 µL of dNTPs (10 mM of each dATP, dTTP, dGTP, and dCTP), 0.25 µL of GoTaq G2 DNA Polymerase (Promega, Madison, WI, 5371), and 33.75 µL of nuclease free water.

The mixture was centrifuged at 14,000 × *g* for 15 sec prior to placement in a T100 Thermal Cycler (Bio-Rad) with the following settings: initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 15 sec, annealing at 60°C for 20 sec, an extension period at 72°C for 90 sec, and a final extension at 72°C for 5 min. Amplification success was determined by electrophoresis of the PCR products in 1% (w/v) agarose gel for 50 min at 70 V.

3.8.2.2.2. DNA Sequencing

DNA fragments were removed from the agarose gel and purified with a DNA Gel Extraction Kit (Micro Kit #K0832, Thermo Fisher, Waltham, MA 02451). After purification, mold samples were sequenced using a 2 μM IST1 primer, and yeast samples were sequenced using a 2 μM NL-1 primer (5'-GCATATCAATAAGCGGAGGAAAAG-3'), which were outsourced to Eurofin Genomics for automated DNA sequencing (Eurofin Genomics, Louisville, KY 40299). Sequencing results were compared using the GenBank (www.ncbi.nlm.nih.gov/blast) local alignment tool, BLAST.

3.9. Statistical Analysis

A 3×4 factorial arrangement within a completely randomized design structure was used to determine the impact of temperature and RH on mite infestations of ham cubes, ham slices, mold growth, water activity and moisture content for each of the control, XG+PG, and CG+PGA+PG net treatments. When significant differences ($P<0.05$) occurred among treatments, Tukey's HSD test was used to separate treatment means ($P<0.05$).

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1. Untreated Nets

4.1.1. Mite Reproduction Assays

When averaged over relative humidity (RH), no differences ($P>0.05$) existed in mite reproduction at each temperature evaluated for both ham cubes and ham slices (Table 4.1). When averaged over temperature, ham cubes that were stored at 75% RH had more mites (313) ($P<0.05$) than the 55 and 85% RH treatments, but did not differ ($P>0.05$) from the 65% RH treatment (Table 4.2). For ham slices, the 85% RH treatment had fewer mites than all other RHs tested.

The significant interaction between temperature and relative humidity ($P<0.05$) indicated that RH impacted mite growth differently at each temperature (Table 4.3). At 24°C, untreated ham cubes had more mites ($P<0.05$) at 65% RH than any other RH tested. Fewer mites ($P<0.05$) were present at 85% RH than the 65 and 75% RH when treated at 28°C. At 32°C, more mites ($P<0.05$) were present at 75% RH than the other RH treatments, and mite numbers did not differ between the 55, 65, and 85% RH treatments ($P>0.05$). For the 55 and 85% RH treatment, there were no differences ($P>0.05$) in mite reproduction at each temperature that was evaluated. When ham cubes were tested at 65% RH, fewer mites ($P<0.05$) were present at 32°C than at 24 and 28°C. At 24°C and 75% RH, there were fewer mites than at 28 and 32°C. These results confirmed previous

results that stated that *T. putrescentiae* mites' optimal conditions for reproduction were between 60 to 75% RH and 20 to 32°C (Mueller et al., 2006). In the current study, there were between 27 and 135 mites on ham cubes in untreated nets at the 55 and 85% RH treatments which was less ($P<0.05$) than the mite count on ham cubes in untreated nets that were evaluated at 75-85% RH reported by Zhang et al. (2017).

For untreated ham slices tested at 24°C, more mites ($P<0.05$) were present at 65% RH than the other RH treatments (Table 4.3). At 28°C, fewer mites were found on untreated hams at 75 and 85% RH than the 55% RH. At 32°C, more mites ($P<0.05$) were present on untreated ham slices that were treated at 75% RH. Controlling the temperature at 32°C reduced ($P<0.05$) mite numbers on untreated ham slices tested at 65% RH when compared to 24 and 28°C. At 55% RH, fewer mites ($P<0.05$) were present on untreated ham slices at 24 and 32°C than 28°C. At 75% RH, more mites ($P<0.05$) were present at 32°C than the other temperatures evaluated, indicating that these were the optimal conditions for mite growth on ham slices.

These results for ham slices were similar to findings reported by Barker (1967). This author stated that the most rapid development of *T. putrescentiae* on dry cured ham occurred at 32.2°C. However, regardless of temperature, the mite count in the current study was maintained below the inoculum level of 50 when untreated nets were used and ham slices were exposed to 85% RH. The lower mite count at 85% RH can potentially be attributed to the greater moisture content of the slices when compared to the ham cubes evaluated at the same RH. Overall, these results indicate that maintaining the dry cured ham aging facility at 85% RH or greater may be more effective at controlling mite

infestations than lesser RHs when untreated nets are used, which are current practices in the U.S. dry cured ham industry.

4.1.2. Water Activity and Moisture Content

The average water activity (A_w) ranged from 0.85 to 0.93 for ham slices for all temperature and relative humidity combinations that were evaluated (Table 4.4). At 24°C, the A_w was less ($P<0.05$) for the 55% RH treatment than the 75 and 85% RH treatments. At 28°C, the A_w of ham slices was greater ($P<0.05$) at 75% RH than ham slices that were stored at 55 and 65% RH, and ham slices at 85% had greater ($P<0.05$) A_w than slices stored at 65% RH (Table 4.4). At 32°C, the 55% RH treatment had less ($P<0.05$) A_w than ham slices stored at all other RHs. For the 55% RH treatment, A_w was less ($P<0.05$) at 32°C than 24 and 28°C. Ham slices stored at 75% RH had less ($P<0.05$) A_w at 32°C when compared to ham slices stored at 24°C.

On average, the moisture content of ham slices ranged from 51 to 65% when evaluated at each temperature and relative humidity combination tested (Table 4.4). The moisture content increased as the RH increased for each temperature, with the exception of the 32°C treatment. In addition, for each temperature, the increase in moisture demonstrated a quadratic response ($P<0.05$) from 55 to 85% RH, and the moisture content of ham slices did not differ ($P>0.05$) at any temperature tested regardless of the RH evaluated, with the exception of the 75% RH treatment. There was less ($P<0.05$) moisture in ham slices at 32°C and 75% RH than at 24 and 28ºC at the same RH treatment.

Overall, these results revealed that greater A_w and moisture content was due to the increase in RH level, which were in accordance with research by Arnau et al. (2003). These authors evaluated the effect of environmental relative humidity during dry cured ham aging and reported the greatest water activity and moisture content at 85% RH and the least water activity at 52% RH. In particular, ham cubes have a larger surface to volume ratio when compared to ham slices, which causes water to evaporate more rapidly and increases the drying rate of the ham cubes. Therefore, the ham cubes were less moist than the ham slices when evaluated at the same temperature and RH. More mites were observed on ham cubes when compared to ham slices. The moisture content and water activity is decreased gradually as time progress in traditional dry cured ham processing to obtain the desired textural and flavor characteristics, which decreases the potential for bacterial growth on the ham surfaces (Borch et al., 1996).

4.1.3. Evaluation of Mold Growth on Ham Surfaces

There was no difference ($P>0.05$) in mold growth on ham slice surfaces when averaged over RHs (Table 4.5). However, the 65% RH had more ($P<0.05$) mold growth than the 55 and 75% RH treatments. Significant interaction between temperature and relative humidity ($P<0.05$) indicated that RH affected mold growth differently for ham slices at each temperature.

In this study, mold growth on ham slices in untreated nets ranged from 0.19 to 61%. When each temperature was compared at each RH treatment, there was no difference ($P>0.05$) in mold growth for the 55, 75, and 85% RH. However, at 65% RH, there was more mold growth ($P<0.05$) at 24°C than 32°C At 65% RH, mold covered 11% of untreated ham slices when evaluated at 32°C. The greatest amount (61%) of mold

growth was present at 24°C and 65% RH. When RH treatments were compared at 24°C, mold growth was greater at 65% RH than the other RH treatments. At 28°C, mold growth was greater at 65% RH in comparison to 75% RH, but no other differences existed. At 32°C, no differences existed among RH with respect to mold growth.

On average, mold growth covered a greater ($P<0.05$) surface area of the ham slices evaluated at 65% RH than any other temperature and RH combination that was evaluated. This corresponded to mite counts of 960 and 520 for the 24 and 28°C treatments at 65% RH. It has previously been hypothesized that the mold functions as a source of shelter and free water for mites (Canfield and Wrenn, 2010). Similar results were obtained in this study via the visual observation of mobile mites and the localization of their eggs near the fungal mycelium on ham slices in untreated nets. In general, fungal growth is desired for the development of the unique aroma, flavor, and texture of dry cured hams. However, some fungal species, further discussed in section 4.10.4, are undesirable via their ability to produce toxins that are harmful to humans, off flavors, and/or off odors. Since untreated nets ultimately did not control mite reproduction and mold growth at different temperature and relative humidity values, an integrated approach via the use of food grade infused nets was evaluated to determine the effect of temperature and RH on *T. putrescentiae* mite infestation on dry cured ham.

4.2. Xanthan Gum + Propylene Glycol Nets

4.2.1. Mite Reproduction Assays

Recent work by Zhao et al. (2016b) evaluated PG at 10 to 50% (w/w) in combination with XG based coatings for their efficacy at preventing *T. putrescentiae* growth. Results indicated that a combination of XG + 20% or more PG was effective at

inhibiting mite growth (Zhao et al., 2016b). Campbell et al. (2016b) successfully infused xanthan gum based coatings into ham nets and provided a more feasible and economical alternative to control the reproduction and development of *T. putrescentiae* mites on dry cured hams. The results of the current study were in agreement with previous findings which revealed that XG + PG nets were more effective than untreated nets when used to control *T. putrescentiae* mite infestations on dry cured hams. For ham cubes stored in XG + PG nets, the ham cubes at 65% RH harbored more mites ($P<0.05$) than all other RHs (Table 4.6), and significant interaction between temperature and RH ($P<0.05$) indicated that RH impacted mite growth differently for ham cubes at each temperature that was evaluated.

No differences existed ($P>0.05$) in mite counts between RH treatments for both ham cubes and slices at 24 and 32°C (Table 4.8). In addition, both ham cube and ham slice mite counts were maintained below the initial inoculum levels of 20 and 50 mites, respectively, with the exception of ham cubes treated at 28°C × 65% RH and ham slices treated at 24°C × 65% RH and 28°C × 65% RH. Complete inhibition of mite growth was also observed at 85% RH when averaged over temperature (Table 4.8). These results indicated that the use of XG + PG nets in aging houses maintained at 85% RH may be more effective at controlling mite reproduction than the lower RHs, regardless of the temperature. These results support the findings of Zhao et al. (2016) in which food grade coatings containing PG completely inhibited mite growth on ham cubes after 14 d of incubation. In addition, results also indicated that mite reproduction can be slowed via the application of XG + PG nets due to each evaluated combination's ability to maintain mite counts that were less than the initial inoculum level of 50 on the ham slices, with the

exception of the 24°C and 28°C temperature treatments at 65% RH. It is important to understand the variability in the temperature and relative humidity threshold for *T. putrescentiae* mite reproduction when studying the effects of these parameters on mite infestations. *T. putrescentiae* mites tend to adapt to unfavorable environments (>80% RH) via acclimation (Sánchez-Ramos and Castañera, 2001). In contrast, this study revealed how *T. putrescentiae* would likely not be able to adapt in the presence of XG + PG nets when treated at 85% RH.

4.2.2. Water Activity and Moisture Content

On average, the A_w of ham slices ranged between 0.86 and 0.93 for all temperature and relative humidity combinations tested (Table 4.9). The A_w was less ($P<0.05$) at 55% RH when the hams slices were stored at 32°C in comparison to 24 and 28°C. At 24°C, the 85% RH treatment had greater A_w ($P<0.05$) than the 55 and 65% RH treatment. Similarly, at 28°C, the 85% RH treatment yielded slices with greater Aw than slices at 65% RH. At 32°C, the A_w of the 55% RH treatment was less than the A_w of ham slices that were exposed to all other RHs.

On average, the moisture content of ham slices in XG + PG nets ranged between 53 and 67% for all temperature and relative humidity combinations. At 85% RH, the moisture content of the hams slices was greater ($P<0.05$) than ham slices that were stored at 55 and 65% RH. Significant interaction between temperature and relative humidity ($P<0.05$) indicated that RH impacted moisture content differently at each temperature. At 55% RH, ham slices that were stored at 28°C had more moisture than ham slices that were stored at 32°C (Table 4.9). At 65% RH, ham slices that were stored at 24°C had less moisture than those stored at 28°C and 32°C.

Results indicated that temperature and RH should be carefully monitored to control mite growth on dry cured hams in XG + PG nets. The use of XG + PG nets followed a similar trend to untreated nets: as temperature increased, a decrease in A_w and moisture content was observed. At lower temperatures, mite reproduction was affected by the increase in A_w and moisture content which has been referenced to changes in the biological functionality of the supracoxal gland of *T. putrescentiae*. The supracoxal gland of ham mites is responsible for capturing moisture from the air and maintaining water balance to help the mites survive (Wharton and Furumizo, 1977; Arlian, 1992). Bodily fluids are lost through the mite's body surface, which makes them susceptible to desiccation. The current study confirms this effect through the observation of *T. putrescentiae* with dried body structures on hams slices treated with XG + PG nets.

4.2.3. Evaluation of Mold Growth on Ham Surfaces

Mold growth on ham slices in XG + PG nets ranged between 0 and 10%, which indicates that the XG+PG treated nets were more effective at preventing mold growth when compared to the untreated nets (Table 4.10). Minimal mold growth on hams treated in XG + PG nets is a result of the antifungal properties of PG, a registered fungistat, that is effective at inhibiting the growth of mold, yeast, and many other microbial containments (EPA, 2006). At 24°C, 85% RH completely inhibited mold growth (Table 4.10), and had less mold than the 65% RH treatment at 24°C. There was no difference ($P>0.05$) in mold growth at each RH when evaluated at 28 and 32°C. In addition, no difference ($P>0.05$) existed in mold growth between temperatures at each RH. In the study, complete inhibition of mold growth was observed at 28°C and 75% RH and 24°C and 85% RH. These results indicate that the 28°C and 75% RH and 24°C and 85% RH

treatment combinations may be the most effective at controlling mold growth on hams in XG + PG nets if they were implemented in the dry-cured ham industry.

4.3. Carrageenan + Propylene Glycol Alginate + Propylene Glycol Nets

4.3.1. Mite Reproduction Assays

The CG + PGA + PG infused nets controlled mite reproduction better than the XG + PG and untreated nets. This confirms previous results that were reported by Zhang et al. (2017). When averaged over RH, mite reproduction on ham cubes was greater ($P<0.05$) at 28°C when compared to 24°C and 32°C (Table 4.11). In addition, more mite growth ($P<0.05$) was observed on ham cubes at 65% RH when compared to 55, 75, and 85% RH (Table 4.12). The significant interaction between temperature and RH ($P<0.05$) indicated that both temperature and RH affected mite growth differently for ham cubes. However, no interaction was present ($P>0.05$) between temperature and RH for mite reproduction on ham slices.

Ham cubes evaluated at 24°C differed ($P<0.05$) in mite growth between 65 and 85% RH (Table 4.13). At 28°C, mite reproduction was greater ($P<0.05$) at 65% RH than at all other RHs. At each temperature evaluated, mite counts did not differ ($P>0.05$) on ham cubes tested at 55, 75, and 85% RH. The 28°C × 65% RH combination had more mites ($P<0.05$) than the other RHs tested, but the mite count (10) was still less than the initial inoculation level of 20.

Fewer mites were observed on ham cubes and slices at all temperature and RH combinations tested in comparison to the untreated and XG + PG net treatments. The maximum average number of mites (31) on ham slices were present at 32°C × 75% RH. These results were similar to Zhang et al, (2017) in which the mite count was 3 of fewer

when CG + PGA + PG nets were evaluated on dry cured ham cubes at 75 to 85% RH. The CG + PGA + PG nets were effective at controlling mite infestations on dry cured hams regardless of temperature and/or relative humidity conditions that were used. However, it was evident that mite reproduction was best controlled at 65% RH and 32°C, 75% RH at 24 and 28°C, and 85% RH at all temperatures since there were zero living mites on these treatments. These results demonstrate how mite reproduction can be controlled via CG + PGA + PG nets, even at favorable conditions for mite production. It was also evident that it may be important to maintain a RH of 85% or greater when the temperature of the aging room is greater than 30°C. Overall, the CG + PGA + PG nets provided a wider range of temperature and RH combinations to control *T. putrescentiae* infestations on dry cured hams in comparison to the untreated and XG + PG nets. This may possibly be due to the differences in the polysaccharide matrix and the heating process involved in CG + PGA + PG net infusion. However, the justification for this finding is currently unknown and will be further investigated.

4.3.2. Water Activity and Moisture Content

Water activity ranged between 0.86 and 0.94 for ham slices in CG + PGA + PG nets that were exposed to various temperature and relative humidity combinations. Similarly to the untreated and XG+PG nets, the A_w decreased with the increase in temperature. (Table 4.14). The A_w of ham slices was greater ($P<0.05$) when stored at 75 and 85% RH in comparison to the 55 and 65% RH treatments for all temperatures tested. At 24°C, the A_w was also less ($P<0.05$) in ham slices that were stored at 55% RH when compared to ham slices that were exposed to 65% RH. At 55% and 75% RH, the A_w was less ($P<0.05$) in ham slices stored at 32°C than at 24 and 28°C. Similarly, at the 65% RH

treatment, the A_w was less ($P<0.05$) at 32°C than at 24°C. At 85% RH, the A_w of the ham slices was greater at 24°C than 28 and 32°C.

There was no difference ($P>0.05$) in moisture content for the tested temperatures when averaged over RHs (Table 4.11). However, differences ($P<0.05$) in moisture content existed at each RH when averaged over temperature (Table 4.12). The moisture content was greater ($P<0.05$) for ham slices evaluated at 85% RH than the 55 and 65% RH treatments. In addition, the 75% RH treated ham slices had greater moisture ($P<0.05$) than the 55% RH treated ham slices. The significant interaction between temperature and relative humidity ($P<0.05$) indicated that RH affected moisture content differently for ham slices at each temperature (Table 4.14). For ham slices treated at 55% RH, there was less moisture at 24°C ($P<0.05$) than the 28 and 32°C treatments. At 65% RH, there were no differences ($P>0.05$) in moisture content among ham slices exposed to different temperatures. At 75% RH, moisture was less at 32°C than at 24°C. At 85% RH, the moisture content of ham slices at 28°C had less ($P<0.05$) moisture than ham slices that were stored at 24°C. The moisture differences between relative humidity treatments was greater at 24°C than the 28 and 32°C treatments. At 24°C, the 75 and 85% RH treatments had greater moisture ($P<0.05$) than the 65 and 55% RH treatments. In addition, the 55% RH treatment had less moisture ($P<0.05$) than all other RH treatments. At 28°C, the 75 and 85% RH treatments had greater ($P<0.05$) moisture than the 55% RH treatment, but no other differences existed ($P>0.05$). At 32°C, the 85% RH treatment had more moisture than all other RH treatments, with no other differences present ($P>0.05$).

4.3.3. Evaluation of Mold Growth on Ham Surfaces

The minimal mold growth on hams treated with CG + PGA + PG nets can be credited to heating the solution of food grade ingredients to approximately 90°C during the production process, which killed any mold that may have been present in the coating ingredients. Mold growth was greater ($P<0.05$) at 28°C than any other temperature when averaged over RHs (Table 4.11). When averaged over temperature, 5% of the slice's surface had mold present when it was exposed to 85% RH, which was greater ($P<0.05$) than the 55 and 75% RH treatments (Table 4.12). Significant interaction between temperature and relative humidity ($P<0.05$) indicated that RH affected mold growth differently for ham slices at each temperature that was evaluated (Table 4.15). At 28°C, more ($P<0.05$) mold was present at 85% RH than the other RHs. Complete inhibition of mold growth was observed at 75% RH × 28°C and 85% RH × 24°C . The same results were observed in XG + PG and there was approximately 1% mold growth found on ham slices in untreated nets at these two temperature and RH combinations. On average, mold growth on ham slices in CG + PGA + PG did not exceed 12% surface coverage when evaluated at any temperature and RH combination.

4.4. Mycological Assessment on Ham Slices

4.4.1. Traditional Identification

Five mold and 2 yeast isolates were obtained and assessed from dry cured ham slices that were incubated for 14 d in untreated and treated nets at different temperature and RH combinations (Table 4.16). The phenotypic traits of the mold and yeast cultures indicates that the isolates belonged to the *Cladosporium, Penicillium* (mold isolates) and *Debaryomyce*s (yeast isolates) genera. These results were similar to previous research

which identified these mold and yeast genera on dry cured ham (Comi et al., 2004; Asefa et al., 2010).

4.4.2. Molecular Identification

The fungus-specific universal primers that were used in this study successfully amplified both mold and yeast DNA by revealing a single, high molecular weight band after gel electrophoresis (Figure 4.1). PCR products for both mold and yeast samples were purified and sequencing analyses resulted in 99 to 100% agreement for each mold and yeast species listed in Table 4.17 (Asefa et al., 2010; Safranek, 2014). One mold sample was collected from a single ham slice in XG + PG nets that was stored at 28°C × 75% RH. *Cladosporium sphaerospermum* was identified on this ham slice. In addition, four ham slices in untreated nets at various temperature and relative humidity combinations was sampled, and molds of the *Penicillium* genus were most frequently present on these ham slices. *Penicillium* species including *P. griseoroseum* (32°C × 75% RH), *P. commune* (24°C × 55% RH), *P. nalgiovense* (28°C × 55% RH), and *P. camemberti* (28°C × 85% RH) were identified in this study. The two yeast samples were identified from two different ham slices in untreated nets. Both yeast isolates were identified as *Debaryomyces hansenii*.

Previous studies indicated that the mold and yeast species that were identified in the current study as the dominant species on dry cured hams during the aging process, which contribute to the development of the ham's aroma, flavor, and texture (Comi et al., 2004; Simoncini et al., 2007; Asefa et al., 2010). For example, the lipolytic activity of *Debaryomyces hansenii* contributes to the aroma and flavor development of dry cured hams via the release of aroma precursors such as oleic and palmitic acids (Simoncini et

al., 2007). The *Penicillium spp.* were most frequently found on surfaces of hams aged at RHs greater than 80% and A_w values greater than 0.90, which are similar to results reported by Comi et al., (2004). However, the growth of *Penicillium spp.* on dry cured ham is commonly unfavorable due to their potential to produce off flavors, off odors and cause health problems for consumers that are allergic to penicillin (Northolt and Bullerman, 1982; Asefa et al., 2009). In particular, Asefa et al. (2010) reported that hams contaminated by *P. commune* harbored a phenol-like off odor that was also observed on ham slice 821 M in this study that was stored at 24ºC and 55% RH (Asefa et al., 2010).

Table 4.1 Mean values for mite infestation on dry cured ham cubes and slices, visible mold growth, water activity, and moisture content of ham slices in untreated nets when averaged over relative humidity post 14 d of incubation.

Temperature (°C)	Mite count on ham cubes (No.)	Mite count on ham slices (No.)	Mold growth (%)	Water activity	Moisture content (%)
24	165	348	19	0.91[A]	59
28	218	396	21	0.91[A]	60
32	147	528	15	0.89[B]	57
SEM	18	55	2.8	0.002	0.54

A-B: means with the same letter by column are not different (P>0.05) due to temperature
SEM: standard error of the mean

Table 4.2 Mean values for mite infestation on dry cured ham cubes and slices, visible mold growth, water activity, and moisture content of ham slices in untreated nets when averaged over temperature post 14 d of incubation.

Relative humidity (%)	Mite count on ham cubes (No.)	Mite count on ham slices (No.)	Mold growth (%)	Water activity	Moisture content (%)
55	91^{B}	456^{A}	10^{B}	0.88^{B}	53^{C}
65	211^{AB}	515^{A}	37^{A}	0.90^{B}	58^{B}
75	313^{A}	696^{A}	8^{B}	0.92^{A}	61^{AB}
85	91^{B}	28^{B}	19^{AB}	0.92^{A}	63^{A}
SEM	18	55	2.8	0.002	0.54

A-B: means with the same letter by column are not different (P>0.05) due to relative humidity

SEM: standard error of the mean

Table 4.3 Mean values for mite infestation on dry cured ham cubes and slices in untreated nets tested at combinations of temperature and relative humidity post 14 d of incubation.

	Temperature (°C)	55% RH	65% RH	75% RH	85% RH	SEM
Mite count on ham cubes (No.)	24	27^{Ab}	365^{Aa}	144^{Bb}	126^{Ab}	37
	28	135^{Abc}	225^{Ab}	467^{Aa}	44^{Ac}	38
	32	111^{Ab}	44^{Bb}	328^{Aa}	105^{Ab}	12
	SEM	36	42	38	32	
Mite count on ham slices (No.)	24	297^{Bb}	957^{Aa}	89^{Bb}	48^{Ab}	93
	28	919^{Aa}	520^{ABab}	107^{Bb}	37^{Ab}	55
	32	153^{Bb}	65^{Bb}	1892^{Aa}	0.33^{Ab}	131
	SEM	49	138	177	11	

A-B: means with the same letter by column within ham cubes or ham slices are not different (P>0.05) due to relative humidity

a-c: means with the same letter by row within ham cubes or ham slices are not different (P>0.05) due to temperature

SEM: standard error of the mean

Table 4.4 Mean values for the water activity and moisture content (%) of dry cured ham slices in untreated nets tested at combinations of temperature and relative humidity post 14 d of incubation.

	Temperature (°C)	55% RH	65% RH	75% RH	85% RH	SEM
Water Activity	24	0.89^{Ab}	0.90^{Aab}	0.92^{ABa}	0.93^{Aa}	0.005
	28	0.90^{Abc}	0.89^{Ac}	0.93^{Aa}	0.92^{Aab}	0.003
	32	0.85^{Bb}	0.89^{Aa}	0.90^{Ba}	0.91^{Aa}	0.005
	SEM	0.008	0.004	0.003	0.003	
Moisture Content (%)	24	51^{Ab}	56^{Ab}	63^{Aa}	65^{Aa}	0.46
	28	56^{Ab}	59^{Aab}	63^{Aa}	62^{Aa}	1.1
	32	52^{Ac}	58^{Aab}	56^{Bbc}	62^{Aa}	1.2
	SEM	0.69	1.3	1.2	1.2	

A-B: means with the same letter by column within water activity or moisture content are not different (P>0.05) due to relative humidity

a-c: means with the same letter by row within water activity or moisture content are not different (P>0.05) due to temperature

SEM: standard error of the mean

Table 4.5 Mean values for visible mold growth (%) on dry cured ham slices in untreated nets tested at combinations of temperature and relative humidity post 14 d of incubation.

Temperature (°C)	55% RH	65% RH	75% RH	85% RH	SEM
24	6.9^{Ab}	61^{Aa}	7.2^{Ab}	0.56^{Ab}	6.2
28	18^{Aab}	38^{ABa}	0.19^{Ab}	27^{Aab}	4.9
32	3.8^{Aa}	11^{Ba}	15^{Aa}	29^{Aa}	3.8
SEM	3	9.8	3.7	4.7	

A-B: means with the same letter by column are not different due ($P>0.05$) to relative humidity

a-b: means with the same letter by row are not different ($P>0.05$) due to temperature

SEM: standard error of the mean

Table 4.6 Mean values for mite infestation on dry cured ham cubes and slices, visible mold growth, water activity, and moisture content of ham slices in nets infused with xanthan gum and propylene glycol when averaged over relative humidity post 14 d of incubation.

Temperature (°C)	Mite count on ham cubes (No.)	Mite count on ham slices (No.)	Mold growth (%)	Water activity	Moisture content (%)
24	1.8	34	3.1	0.92^{A}	60
28	2.6	19	4.3	0.92^{A}	61
32	1.1	5	3.1	0.86^{B}	60
SEM	0.28	11	0.82	0.003	0.49

A-B: means with the same letter by column are not different (P>0.05) due to temperature
SEM: standard error of the mean

Table 4.7 Mean values for mite infestation on dry cured ham cubes and slices, visible mold growth, water activity, and moisture content of ham slices in nets infused with xanthan gum and propylene glycol when averaged over temperature post 14 d of incubation.

RH (%)	Mite count on ham cubes (No.)	Mite count on ham slices (No.)	Mold growth (%)	Water activity	Moisture content (%)
55	1.1^{B}	12^{A}	1.8^{AB}	0.89^{B}	56^{C}
65	4.1^{A}	66^{A}	7.7^{A}	0.90^{B}	59^{BC}
75	0.67^{B}	0.33^{A}	0.56^{B}	0.92^{A}	61^{AB}
85	1.3^{B}	0^{A}	3.8^{AB}	0.92^{A}	64^{A}
SEM	0.28	11	0.82	0.003	0.49

A-C: means with the same letter by column are not different (P>0.05) due to relative humidity

SEM: standard error of the mean

Table 4.8 Mean values for mite infestation on dry cured ham cubes and slices in nets infused with xanthan gum and propylene glycol tested at combinations of temperature and relative humidity post 14 d of incubation.

	Temperature (°C)	55% RH	65% RH	75% RH	85% RH	SEM
Mite count on ham cubes (No.)	24	0.67^{Aa}	3.0^{Ba}	1.3^{Aa}	2.0^{Aa}	0.58
	28	0.33^{Ab}	9.0^{Aa}	0.33^{Ab}	0.67^{Ab}	0.43
	32	2.3^{Aa}	0.33^{Ba}	0.33^{Aa}	1.3^{Aa}	0.48
	SEM	0.46	0.77	0.20	0.73	
Mite count on ham slices (No.)	24	9^{Ab}	128^{Aa}	0^{Ab}	0^{Ab}	32
	28	20^{Aa}	54^{ABa}	0.67^{Aa}	0^{Aa}	7.2
	32	6.3^{Aa}	15^{Ba}	0.33^{Aa}	0^{Aa}	3.9
	SEM	5.1	44	0.26	0	

A-B: means with the same letter by column within ham cubes or ham slices are not different (P>0.05) due to relative humidity

a-b: means with the same letter by row within ham cubes or ham slices are not different (P>0.05) due to temperature

SEM: standard error of the mean

Table 4.9 Mean values for the water activity and moisture content (%) of dry cured ham slices in nets infused with xanthan gum and propylene glycol tested at combinations of temperature and relative humidity post 14 d of incubation.

	Temperature (°C)	55% RH	65% RH	75% RH	85% RH	SEM
Water Activity	24	0.90^{Ab}	0.91^{Ab}	0.92^{Aab}	0.93^{Aa}	0.006
	28	0.91^{Aab}	0.90^{Ab}	0.93^{Aa}	0.93^{Aa}	0.004
	32	0.86^{Bb}	0.89^{Aa}	0.91^{Aa}	0.91^{Aa}	0.003
	SEM	0.009	0.004	0.003	0.003	
Moisture Content (%)	24	54^{Bb}	56^{Bb}	63^{Aa}	67^{Aa}	0.97
	28	60^{Aa}	60^{ABa}	62^{Aa}	62^{Aa}	0.93
	32	53^{Bb}	62^{Aa}	60^{Aa}	64^{Aa}	0.72
	SEM	1.67	0.50	0.55	0.97	

A-B: means with the same letter by column within water activity or moisture content are not different (P>0.05) due to relative humidity
a-b: means with the same letter by row within water activity or moisture content are not different (P>0.05) due to temperature
SEM: standard error of the mean

Table 4.10 Mean values for visible mold growth (%) on dry cured ham slices in nets infused with xanthan gum and propylene glycol tested at combinations of temperature and relative humidity post 14 d of incubation.

Temperature (°C)	55% RH	65% RH	75% RH	85% RH	SEM
24	1.9^{Aab}	9.2^{Aa}	1.1^{Aab}	0^{Ab}	1.1
28	3.2^{Aa}	6.7^{Aa}	0^{Aa}	7.3^{Aa}	1.6
32	0.38^{Aa}	7.2^{Aa}	0.56^{Aa}	4.2^{Aa}	1.7
SEM	0.51	2.4	0.23	2.4	

A: means with the same letter by column are not different (P>0.05) due to relative humidity

a-b: means with the same letter by row are not different (P>0.05) due to temperature

SEM: standard error of the mean

Table 4.11 Mean values for mite infestation on dry cured ham cubes and slices, visible mold growth, water activity, and moisture content of ham slices in nets infused with carrageenan, propylene glycol alginate, and propylene glycol when averaged over relative humidity post 14 d of incubation.

Temperature (°C)	Mite count on ham cubes (No.)	Mite count on ham slices (No.)	Mold growth (%)	Water activity	Moisture content (%)
24	1.3^{B}	1.3	0.95^{B}	0.92^{A}	60
28	3.1^{A}	3.2	4.4^{A}	0.91^{A}	60
32	0.08^{B}	9.5	1.66^{B}	0.89^{B}	58
SEM	0.24	2.69	0.41	0.002	0.47

A-B: means with the same letter by column are not different (P>0.05) due to temperature
SEM: standard error of the mean

Table 4.12 Mean values for mite infestation on dry cured ham cubes and slices, visible mold growth, water activity, and moisture content of ham slices in nets infused with carrageenan, propylene glycol alginate, and propylene glycol when averaged over temperature post 14 d of incubation.

RH (%)	Mite count on ham cubes (No.)	Mite count on ham slices (No.)	Mold growth (%)	Water activity	Moisture content (%)
55	0.33^{B}	6.3^{A}	1.5^{B}	0.88^{B}	55^{C}
65	4.4^{A}	2^{A}	2^{AB}	0.89^{B}	58^{BC}
75	1.1^{B}	10^{A}	1^{B}	0.92^{A}	61^{AB}
85	0.11^{B}	0^{A}	5^{A}	0.92^{A}	63^{A}
SEM	0.24	2.69	0.41	0.002	0.47

A-C: means with the same letter by column are not different (P>0.05) due to relative humidity

SEM: standard error of the mean

Table 4.13 Mean values for mite infestation on dry cured ham cubes and slices in nets infused with carrageenan, propylene glycol alginate, and propylene glycol tested at combinations of temperature and relative humidity post 14 d of incubation.

	Temperature (°C)	55% RH	65% RH	75% RH	85% RH	SEM
Mite count on ham cubes (No.)	24	0.33^{Aab}	2.7^{Ba}	2.3^{Aab}	0^{Ab}	0.43
	28	0.67^{Ab}	10^{Aa}	1^{Ab}	0.33^{Ab}	0.58
	32	0^{Aa}	0.33^{Ba}	0^{Aa}	0^{Aa}	0.09
	SEM	0.26	0.89	0.31	0.12	
Mite count on ham slices (No.)	24	0.67^{Aa}	4.3^{Aa}	0^{Ba}	0^{Aa}	1
	28	11^{Aa}	1.7^{Aa}	0^{Ba}	0^{Aa}	2.9
	32	7.3^{Aab}	0^{Ab}	31^{Aa}	0^{Ab}	7.7
	SEM	4.7	1.4	10	0	

A-B: means with the same letter by column within ham cubes or ham slices are not different (P>0.05) due to relative humidity

a-b: means with the same letter by row within ham cubes or ham slices are not different (P>0.05) due to temperature

SEM: standard error of the mean

Table 4.14 Mean values for the water activity and moisture content (%) of dry cured ham slices in nets infused with carrageenan, propylene glycol alginate, and propylene glycol tested at combinations of temperature and relative humidity post 14 d of incubation.

	Temperature (°C)	55% RH	65% RH	75% RH	85% RH	SEM
Water Activity	24	0.89^{Ac}	0.91^{Ab}	0.93^{Aa}	0.94^{Aa}	0.004
	28	0.90^{Ab}	0.90^{ABb}	0.93^{Aa}	0.92^{Ba}	0.003
	32	0.86^{Bb}	0.88^{Bb}	0.90^{Ba}	0.91^{Ba}	0.004
	SEM	0.004	0.005	0.002	0.003	
Moisture Content (%)	24	52^{Bc}	58^{Ab}	64^{Aa}	66^{Aa}	0.71
	28	58^{Ab}	59^{Aab}	63^{ABa}	60^{Bab}	0.81
	32	55^{ABb}	56^{Ab}	58^{Bb}	63^{ABa}	0.99
	SEM	0.87	1.1	0.84	1.1	

A-C: means with the same letter by column within water activity or moisture content are not different (P>0.05) due to relative humidity

a-c: means with the same letter by row within water activity or moisture content are not different (P>0.05) due to temperature

SEM: standard error of the mean

Table 4.15 Mean values for visible mold growth (%) on dry cured ham slices in nets infused with carrageenan, propylene glycol alginate, and propylene glycol tested at combinations of temperature and relative humidity post 14 d of incubation.

Temperature (°C)	55% RH	65% RH	75% RH	85% RH	SEM
24	2.2^{Aa}	1.0^{Aa}	0.56^{Aa}	0^{Ba}	0.55
28	1.5^{Ab}	3.7^{Ab}	0^{Ab}	12^{Aa}	0.88
32	0.69^{Aa}	1.2^{Aa}	2.5^{Aa}	2.3^{Ba}	0.74
SEM	0.71	0.67	0.69	1.2	

A-B: means with the same letter by column are not different (P>0.05) due to relative humidity

a-b: means with the same letter by row are not different (P>0.05) due to temperature

SEM: standard error of the mean

Table 4.16 Morphology and molecular differentiation of fungal growth on dry cured hams in untreated or treated (xanthan gum and propylene glycol) nets at 24 to 32°C and 55 to 85% relative humidity combinations.

Sample	Treatment/Net Formulation	Macroscopic Characteristics				Microscopic Characteristics
		Color (top/bottom)	Size (cm)	Form/Elevation	Margin/Surface	
915 M	28°C × 75% RH XG+PG	Tan/Dark green	2.5 cm	Irregular/Raised	Erose/Dull, rough	Conidiospores, branched conidial chains, septate
930 M	32°C × 75% RH untreated	Yellow, green/Yellow	3.8 cm	Circular/Nmbonate	Undulate/Dull, rough	Conidiospores, divergent branches, subglobose conidia
821 M	24°C × 55% RH untreated	Green/Pale yellow	3.8 cm	Circular/Flat	Undulate/Dull, smooth	Terverticillate Conidiospores, subglobose conidia
582 M	28°C × 55% RH untreated	Green, white/Orange	2.5 cm	Circular/Raised, crateriform	Undulate/Glistening, smooth	Conidiospores, branched, flask shaped phialides, globose conidia
238 M	28°C × 85% RH untreated	White/Tan	1.3 cm	Irregular/Flat	Undulate/Dull, rough	Terverticillate conidiospores, tangled chain globose conidia
413 Y	28°C × 75% RH untreated	White/White	Punctiform	Circular/Raised	Entire/Smooth	Multilateral budding, ascus
861 Y	24°C × 55% RH untreated	White/White	Punctiform	Circular/Raised	Entire/Smooth	Multilateral budding, ascus

Size: diameter of mold growth 5 d post inoculation, diameter of yeast growth 3 d post inoculation
M: mold sample and Y: yeast sample

Table 4.17 Molecular identification results via polymerase chain reaction and sequencing of fungal growth on dry cured hams in untreated or treated (xanthan gum and propylene glycol) nets at 24 to 32°C and 55 to 85% relative humidity combinations.

Sample	PCR product (bp)	ITS1 (bp)	Highest Blast hit	Identity (%)
915 M	600	473	*Cladosporium sphaerospermum*	100%
930 M	625	515	*Penicillium griseoroseum*	100%
821 M	625	348	*Penicillium commune*	100%
582 M	625	523	*Penicillium nalgiovense*	100%
238 M	675	294	*Penicillium camemberti*	99%
413 Y	950	527	*Debaryomyces hansenii*	100%
861 Y	950	545	*Debaryomyces hansenii*	100%

bp: base pairs; the estimated total size of PCR product and estimated length size for the ITS1 region

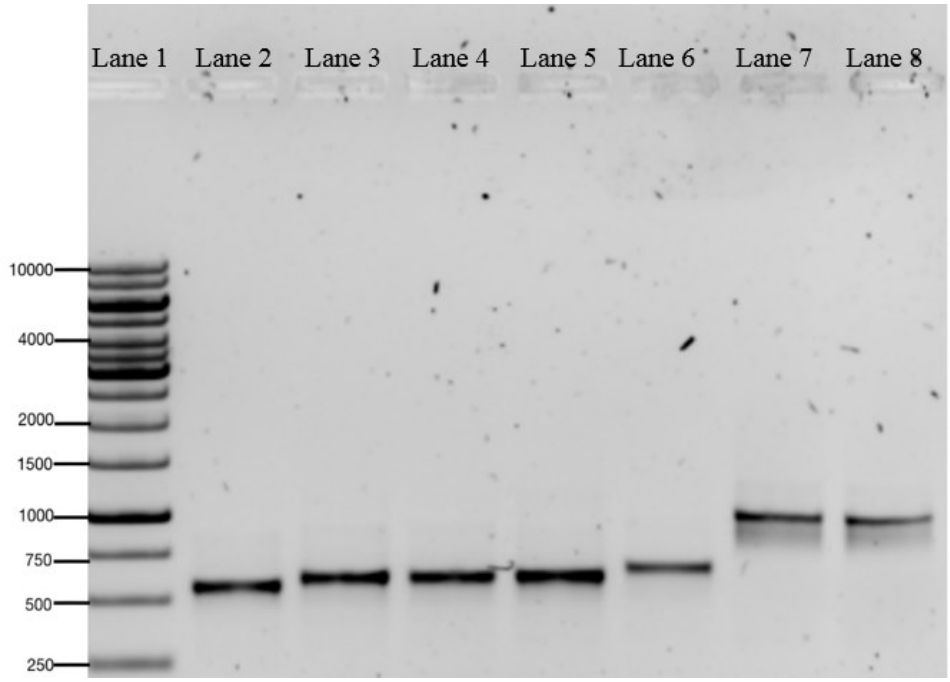

Figure 4.1 Polymerase chain reaction (PCR) amplification of rDNA from mold and yeast genomic DNA.

Lane 1: 1 Kb DNA Ladder; Lanes 2-8: Mold or Yeast Isolates; Lane 2: 915 M; Lane 3: 930 M; Lane 4: 821 M; Lane 5: 582 M; Lane 6: 238 M; Lane 7: 413 Y; Lane 8: 861Y

CHAPTER V

CONCLUSIONS

Mite reproduction was not controlled at any temperature and RH combination in the untreated nets, other than 32°C × 85% RH. The XG+PG net treatments inhibited mite reproduction and minimized mold growth on dry cured ham slices at all tested conditions with the exception of 24°C × 65% RH and 28°C × 65% RH. The CG+PGA+PG net treatments inhibited mite reproduction and mold growth on dry cured ham cubes and slices at all conditions that were tested, with the 85% RH treatments showing the greatest level of inhibition. In addition, treated nets are recommended for use to minimize the growth of the fungal genera *Cladosporium, Penicillium,* and *Debaryomyces* on dry cured hams. Further research is recommended to test the efficacy of treated nets at controlling mite reproduction and mold growth in commercial settings at the temperature and relative humidity combinations that were most effective at controlling mite reproduction and mold growth in the current study.

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